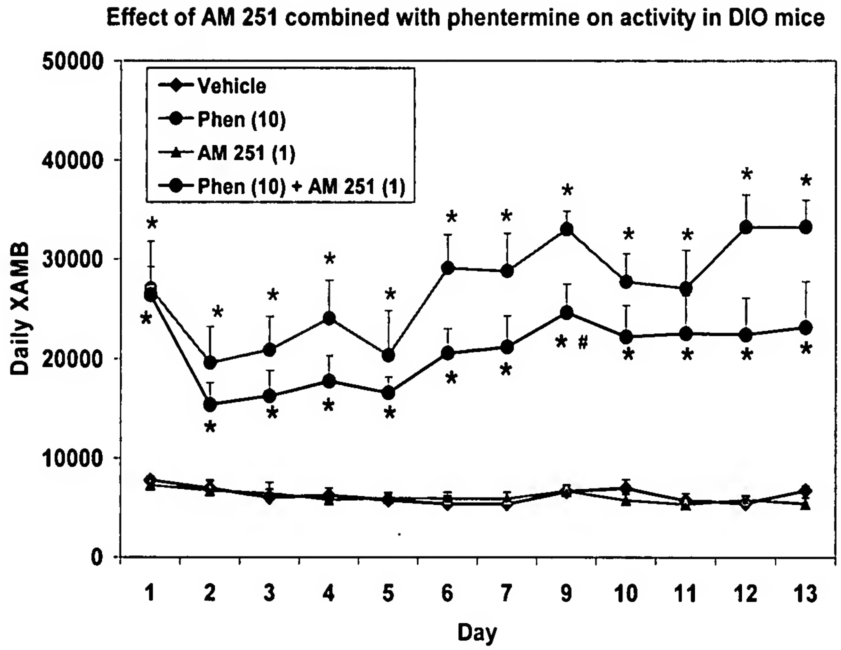

Exhibit 4. Locomotor activity of Phentermine, AM251 and the combination. Both the phentermine (phen) group and the phentermine + AM251 group had increased locomotor activity relative to the vehicle group on all days. On days 6, 9, 12 and 13, the locomotor activity ($p < 0.05$) was decreased in the combination dosing group relative to phentermine alone.

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Comparison of the Effects of Sibutramine and Other Weight-Modifying Drugs on Extracellular Dopamine in the Nucleus Accumbens of Freely Moving Rats

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KEY WORDS sibutramine; weight-reducing drugs; dopamine; nucleus accumbens; in vivo microdialysis

ABSTRACT The acute effects of systemic administration of the anti-obesity agent sibutramine on extracellular dopamine (DA) in the nucleus accumbens of freely moving rats were studied using in vivo microdialysis and compared with the actions of phentermine and *d*-amphetamine at doses 1× and 3× their respective 2 h ED_{50} values to reduce food intake in rats. At the lower dose, sibutramine did not elevate extracellular DA concentrations; however, at the higher dose (6.0 mg kg^{-1}, i.p.) it caused a modest and prolonged increase in extraneuronal DA. A maximal rise was observed at 60 min post-sibutramine treatment (+231% compared to controls) with DA levels remaining elevated for up to 160 min post treatment. In contrast, phentermine and *d*-amphetamine significantly enhanced DA efflux at both the lower and higher doses. These elevations of DA levels were significantly greater than that seen with the corresponding dose of sibutramine over 0–80 min post treatment. Maximal rises in DA levels resulting from the higher dose of each drug were +733% (phentermine, 3.9 mg kg^{-1}, i.p.) and +603% (*d*-amphetamine, 1.5 mg kg^{-1}, i.p.) compared to controls 40 min post treatment. The highest doses of phentermine and *d*-amphetamine increased rat locomotor activity up to 100 min and 160 min post treatment, respectively, whereas the equivalent sibutramine dose had no effect. These findings therefore suggest that dopaminergic reward mechanisms are not involved in the reduction of food intake by sibutramine. Furthermore, they are consistent with the view that sibutramine lacks abuse potential. **Synapse 38: 167–176, 2000.** © 2000 Wiley-Liss, Inc.

INTRODUCTION

Sibutramine (*N*-1-[1-(4-chlorophenyl)cyclobutyl]-3-methybutyl-*N,N*-dimethylamine hydrochloride monohydrate) is a novel noradrenaline (NA) and 5-hydroxytryptamine (5-HT) uptake inhibitor which was initially developed as a potential antidepressant that would have a rapid onset of clinical efficacy by virtue of its ability to rapidly downregulate β-adrenoceptors in the central nervous system (CNS) (Buckett et al., 1988). Although in two large, placebo-controlled, dose-ranging clinical trials sibutramine failed to demonstrate antidepressant efficacy, significant weight loss was unexpectedly observed in the sibutramine-treated patients (Kelly et al., 1995). In the light of this finding, development of sibutramine was switched from depression to obesity, where long-term efficacy has been demonstrated in several placebo-controlled, double-blind clinical trials (Apfelbaum et al., 1999; Bray et al., 1999; Weintraub et al., 1991). Animal studies have shown that sibutramine produces weight loss by actions on both sides of the "energy balance" equation, i.e., to reduce food intake (Jackson et al., 1997a,b) and to increase energy expenditure (Connoley et al., 1999). Experiments using nisoxetine and fluoxetine, which are selective uptake inhibitors of NA and 5-HT, respectively, given both alone and in combination, and those using selective monoaminergic receptor antagonists support the view that sibutramine's effects on food intake and thermogenesis are mediated via potent uptake inhibition of NA and 5-HT in the CNS (Connoley et al., 1999; Jackson et al., 1997a,b). Consistent with

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this hypothesis, microdialysis studies in freely moving rats have demonstrated that at pharmacologically relevant doses sibutramine evokes increases in extracellular concentrations of both NA (Wortley et al., 1999a) and 5-HT (Gundlah et al., 1997; Prow et al., 1997) in the hypothalamus, an area known to be involved in the regulation of food intake and energy expenditure (Leibowitz et al., 1988; Rothwell, 1994). In vitro, sibutramine's pharmacologically active metabolites (Metabolite 1, BTS 54 354, *N*-{-[1-(4-chlorophenyl)cyclobutyl]-3-methylbutyl}-*N*-methylamine hydrochloride and Metabolite 2, BTS 54 505, 1-[1-(4-chlorophenyl)cyclobutyl]-3-methylbutylamine hydrochloride; Luscombe et al., 1989) are uptake inhibitors not only of NA and 5-HT, but also dopamine (DA) in both rat and human brain tissue (Heal et al., 1998a).

Dopaminergic neuronal transmission in the limbic system is of particular importance due to its fundamental involvement in the rewarding and reinforcing properties of drugs of abuse (Di Chiara and Imperato, 1988; Di Chiara et al., 1992), as well as its key role in the behavioural arousal and increased locomotor activity responses to psychostimulant drugs of abuse (Jackson et al., 1975; Kelly et al., 1975). As such, this issue was extensively examined during sibutramine's development as an antidepressant, where its actions as a DA uptake inhibitor in vivo were shown to occur at doses >10-fold greater than those active in animal models predictive of antidepressant efficacy (Heal et al., 1992). However, the relationship between the anti-obesity actions of sibutramine and its effects on central dopaminergic function have not been investigated to date.

Thus, the present study used the technique of in vivo microdialysis to determine the effect of sibutramine on extracellular DA concentrations in the nucleus accumbens of freely moving rats at its ED_{50} dose to reduce food intake at 2 h and at the much higher dose of 3× this value. Pharmacologically equivalent doses of phentermine and *d*-amphetamine were used as comparators in this study for two reasons. First, both drugs have been approved for use as anti-obesity agents and, second, phentermine, *d*-amphetamine, and sibutramine all contain the β-phenylethylamine substructure.

The second aim of the study was to establish whether there was an association between the actions of these drugs on limbic DA function and their psychostimulant potential. This objective was achieved by determining the effects of the higher dose of the three drugs, ie, 3× ED_{50} to inhibit food intake at 2 h, on rat locomotor activity.

MATERIALS AND METHODS

Measurement of [^{3}H]DA uptake into synaptosomes

Tissue preparation

Male Sprague-Dawley rats (Charles River, 220 g) were killed and the nucleus accumbens (~30 mg) rapidly dissected from both hemispheres, homogenised with a motor-driven (Heidolph RZR50 Stirrer) Teflon pestle (12 strokes, 800 rpm, difference in diameter between mortar and pestle 0.5 mm) in ice-cold 0.32 M sucrose (1:10 w/v) and centrifuged at 1,500*g* for 10 min. The pellet (P1) was discarded and the supernatant layer recentrifuged at 30,000*g* for 10 min. The crude synaptosomal pellet (P2) was resuspended in Krebs-Henseleit buffer (126.5 mM NaCl, 27.5 mM $NaHCO_3$, 2.4 mM KCl, 0.5 mM $MgCl_2$, 0.5 mM Na_2SO_4, 1.1 mM $CaCl_2$ and 5.6 mM glucose, adjusted to pH 7.4 at 25°C with 1 M NaOH) equivalent to 4.2 mg wet weight of tissue ml^{-1}. All centrifugations were carried out at 4°C.

Assay

Crude synaptosomes were incubated in a shaking water bath at 37°C (80 oscillations min^{-1}). Aliquots (150 μl; equivalent to 0.625 mg wet weight) of tissue were then added to tubes (1.4 ml Macrowell tube-stripes) containing 275 μl Krebs-Henseleit buffer and either 50 μl of distilled water to define total uptake, 50 μl of GBR 12909 (to define nonspecific uptake; final concentration 10 μM) or 50 μl of test compound at 10 concentrations. Uptake was initiated by the addition of 25 μl of freshly prepared [^{3}H]DA (final concentration 2.5 nM) followed by vortexing and was continued for 5 min at 37°C in the shaking water bath. Uptake was terminated by filtration under vacuum through Skatron 11734 filtermats using a Skatron cell harvester. Filters were then washed with 8 ml ice-cold 0.154 M saline (wash 1,2 at settings 9,9). Scored filter paper discs were punched out into 4.5 ml plastic scintillation vials and 25 μl of [^{3}H]DA were pipetted into four vials for the accurate determination of the concentration added to each tube. Ultima Gold MV scintillation fluid (1.0 ml) was then added to each vial. Radioactivity (dpm) was determined by liquid scintillation counting for 3 min (1900 CA Tri-carb Liquid Scintillation Analyser).

Statistical analysis

The concentration of drug required to inhibit uptake of [^{3}H]DA by 50% (IC_{50}) was calculated using the EBDA iterative curve-fitting program (McPherson, 1983). These data were then converted to inhibition constants (K_i) values using the Cheng and Prusoff (1973) equation.

Feeding studies

Animals and environment

Experiments were performed on male Sprague-Dawley rats (350–500 g at the start of the experiment) which were obtained from Charles River (Margate, UK). Animals were individually housed in polypropylene cages with metal grid floors at a temperature and humidity of 21 ± 1°C and 55%, respectively.

Polypropylene trays with cage pads were placed beneath each cage to detect any food spillage. The animals were maintained on a reverse-phase light-dark cycle. Lights were off from 09.30–17.30 h, during which time the laboratory was illuminated by red lamps. Animals had access to a standard powdered rat diet and tap water at all times. The powdered diet was contained in glass feeding jars (10 cm diameter, 8 cm deep; Solmedia Laboratory Supplies, Romford, UK) with aluminium lids. Each lid had a hole (3 cm diameter) cut in it to allow the rats access to the food. Spillage of powdered diet from the feeding jars was negligible. Animals were acclimatised to these conditions for at least 2 weeks before experimentation began.

Experimental procedures

On the test day, animals were randomly allocated to four different treatment groups. Each treatment group contained 6–8 rats. All procedures began at the onset of the dark phase, since rats consume most of their food intake during the nocturnal period. Feeding jars were weighed (to the nearest 0.1 g on a Sartorius top-pan balance) at the time of drug administration and after 2 h. Each experiment included a vehicle-treated control group and three drug-treated groups. The food intake of animals in the four different treatment groups was monitored concurrently. Variations in body weight were accounted for by expressing food intake in terms of g kg^{-1} rat weight. Animals were then divided into groups at random and reused in the feeding studies after a wash-out period of at least 7 days.

Drugs

Sibutramine hydrochloride monohydrate (Knoll Pharmaceuticals, UK), phentermine hydrochloride, and *d*-amphetamine sulphate (Sigma Chemical Co., UK) were dissolved in 0.154 M saline and administered by i.p. injection using a dose volume of 1.0 ml kg^{-1} body weight. Drug doses are expressed as the free base.

Statistical analysis

ED_{50} values (the dose of drug required to reduce food intake to 50% of control levels in the 2 h following drug administration) were calculated from logistic sigmoid curves with maximum at the control mean and minimum at 0. The curve was fitted by least squares (Marquardt's compromise method) using the computer programme PROC NLIN in SAS.

Microdialysis experiments

Animals and environment

Adult, male CD rats (250–350 g; Charles River) were used. Prior to the experiment, rats were housed in pairs with a 12/12 h light/dark cycle (lights on at 06:00), an ambient temperature of 21°C and 55% humidity. Food and water were available ad libitum.

Surgery and microdialysis

Rats were anaesthetised with isoflurane (5% to induce, 2% to maintain) in an O_2/N_2O mixture (1 l min^{-1} each) delivered via an anaesthetic unit (St. Bernard Medical Services) and a concentric microdialysis probe (300 μm outer diameter) with 2 mm exposed Hospal membrane tip (manufactured in-house) was stereotaxically implanted into the nucleus accumbens (coordinates: A: +2.2 mm; L: -1.5 mm relative to bregma; V: -8.0 mm relative to the skull surface; Paxinos and Watson, 1986). Two additional burr holes were made for skull screws (stainless steel) and the probe was secured using dental cement. Following surgery, animals were individually housed in circular chambers (450 mm internal diameter, 320 mm wall height) with the microdialysis probe connected to a liquid swivel and a counterbalanced arm to allow unrestricted movement. Rats were allowed a recovery period of at least 16 h with food and water available ad libitum and probes were continuously perfused with an artificial cerebrospinal fluid (aCSF; Harvard Apparatus, Dover, MA) of the following electrolyte composition (in mM): sodium 150; potassium 3.0; magnesium 0.8; calcium 1.4; phosphorus 1.0; chloride 155.0. A flow rate of 1.2 μl min^{-1} was used and samples were collected from freely moving rats at 20-min intervals into Eppendorf vials.

DA analysis

Detection and subsequent quantification of DA in the dialysis samples involved the use of reverse-phase, ion-pair HPLC coupled with electrochemical detection. Briefly, the method employed a Spherisorb (100 × 2.1 mm internal diameter; Higgins Analytical) reverse-phase column packed with 3 μm ODS2 material. A Bischoff solvent delivery pump was used to circulate mobile phase (100 mM sodium dihydrogen orthophosphate, 1.0 mM EDTA, 1.0 mM 1-octane sulphonic acid, 12% methanol, pH 4.0) at a flow rate of 0.2 ml min^{-1}. Samples (20 μl) were injected onto the column via a refrigerated (4°C) Triathlon autosampler. An Antec electrochemical detector was used in conjunction with an Antec "wall-jet" design cell (VT 03). The cell employs a high-density, glassy carbon working electrode (+0.65 V) combined with an Ag/AgCl reference electrode. The electrode signal was integrated using a Turbochrom data acquisition system (Perkin-Elmer, Oak Brook, IL). A stock solution of DA (1.0 mM) was prepared by dissolving it in a mixture of equal quantities of deionised water and 0.1 M perchloric acid and stored at 4°C. A working solution was prepared daily.

Pharmacological treatment

Five basal samples were taken prior to a single i.p. injection of either drug (sibutramine, phentermine,

d-amphetamine) or 0.154 M saline. Dialysis samples were collected for 4 h post injection. All drugs were administered at a dose of 1× and 3× their 2 h ED_{50} values, determined from the feeding studies (see Table II) and all doses are expressed as free base. Drugs were prepared by dissolution in 0.154 M saline and administered in a volume of 2.0 ml kg^{-1} of body weight.

Histology

At the end of the experiments, rats were killed and their brains rapidly removed and stored in 10% formaldehyde solution for a minimum of 5 days. Sections (100 μm) were cut on a microtome and mounted on slides. Probe placements were visualised and localised with reference to a stereotaxic atlas (Paxinos and Watson, 1986). Data are reported only from animals where probe membranes were correctly positioned in the nucleus accumbens.

Reagents

All reagents used in HPLC analysis were of HPLC grade. Sodium dihydrogen orthophosphate, 1-octane sulphonic acid, methanol, and 10% formaldehyde solution were obtained from Fisher Scientific (UK). EDTA was from BDH Chemicals Ltd. (UK). DA was purchased from Sigma Chemical Co. (UK).

Statistical analysis

In all experiments, the average of the five pre treatment DA levels were used as a measure of basal levels. All values are mean ± SEM for n = 8 (drug-treated) or 11 (saline-treated) rats. Statistical analysis of individual time points was made by analysis of covariance (ANCOVA) with baseline as the covariate and treatment as the factor, on log-transformed data. Comparisons between drug- and saline-treated groups were carried out using a *t*-test for multiple comparisons. Area under the curve analysis was used to compare differences between sibutramine- and comparator drug-treated groups. A *P* value of <0.05 was considered statistically significant.

Locomotor activity experiments

Animals and environment

Adult, male CD rats (250–350 g; Charles River) were used. Rats were housed in pairs with a 12/12 h light/dark cycle (lights on at 06:00), an ambient temperature of 21°C, and 55% humidity. Food and water were available ad libitum. Sixteen hours prior to the experiment, rats were rehoused singly in the same conditions as above to mimic their individual housing post surgery in microdialysis experiments.

Pharmacological treatment

On the day of the experiment, rats were transferred to the test room and placed randomly into individual clear-sided, perspex activity test cages (480 × 210 × 230 mm). Rats were allowed to acclimatise to these conditions for 1 h prior to a single i.p. injection of either sibutramine, phentermine, *d*-amphetamine, or 0.154 M saline. Locomotor activity was scored automatically by infrared detection beams at 10-min intervals for 3 h post treatment. All test drugs were administered at a dose of 3× their 2 h ED_{50} values (Table II) and all doses are expressed as free base. Drugs were prepared as previously described for microdialysis experiments.

TABLE I. [^{3}H]DA uptake inhibition profile of sibutramine, its metabolites, and other weight-reducing drugs in synaptosomes prepared from rat nucleus accumbens

Drug	K_i (nM)	Hill slope
Sibutramine	542 ± 13	0.8 ± 0.1
Metabolite 1	17 ± 2	0.9 ± 0.2
Metabolite 2	26 ± 4	1.1 ± 0.1
Phentermine	519 ± 34	1.0 ± 0.1
d-Amphetamine	78 ± 7	1.0 ± 0.1

Values are mean ± SEM of three independent determinations.

TABLE II. ED_{50} values for sibutramine and other weight-reducing drugs obtained from acute feeding studies

Drug	Dose (mg kg^{-1}, i.p.)	2 h ED_{50}	95% confidence intervals
Sibutramine	1.0, 3.0, 10.0	2.0	1.0–4.1
Phentermine	0.3, 1.0, 3.0	1.3	0.7–2.5
d-Amphetamine	0.3, 1.0, 3.0	0.5	0.4–0.8

Each treatment group contained 6–8 rats.

Statistical analysis

Mean activity counts for each drug-treated group were determined at 20-min intervals. All values are mean ± SEM (n = 8 rats). The square root transformation was applied to the data, which were then analysed by two-way ANOVA with treatment and day as factors. The least significant difference test was used to compare each drug-treated group to saline-treated controls along with phentermine- and *d*-amphetamine-treated to sibutramine-treated rats. A *P* value of <0.05 was considered statistically significant.

RESULTS

Effects on [^{3}H]DA uptake

The [^{3}H]DA uptake inhibition profiles of sibutramine, its metabolites, and other weight-reducing agents in synaptosomes prepared from rat nucleus accumbens are shown in Table I. The results demonstrate that sibutramine and phentermine are extremely weak inhibitors, whereas *d*-amphetamine and sibutramine's metabolites are moderately potent inhibitors of [^{3}H]DA uptake in vitro.

Feeding studies

The effects of sibutramine and comparator anti-obesity drugs on food intake at 2 h in freely feeding rats during the dark phase are shown in Figure 1. The ED_{50}

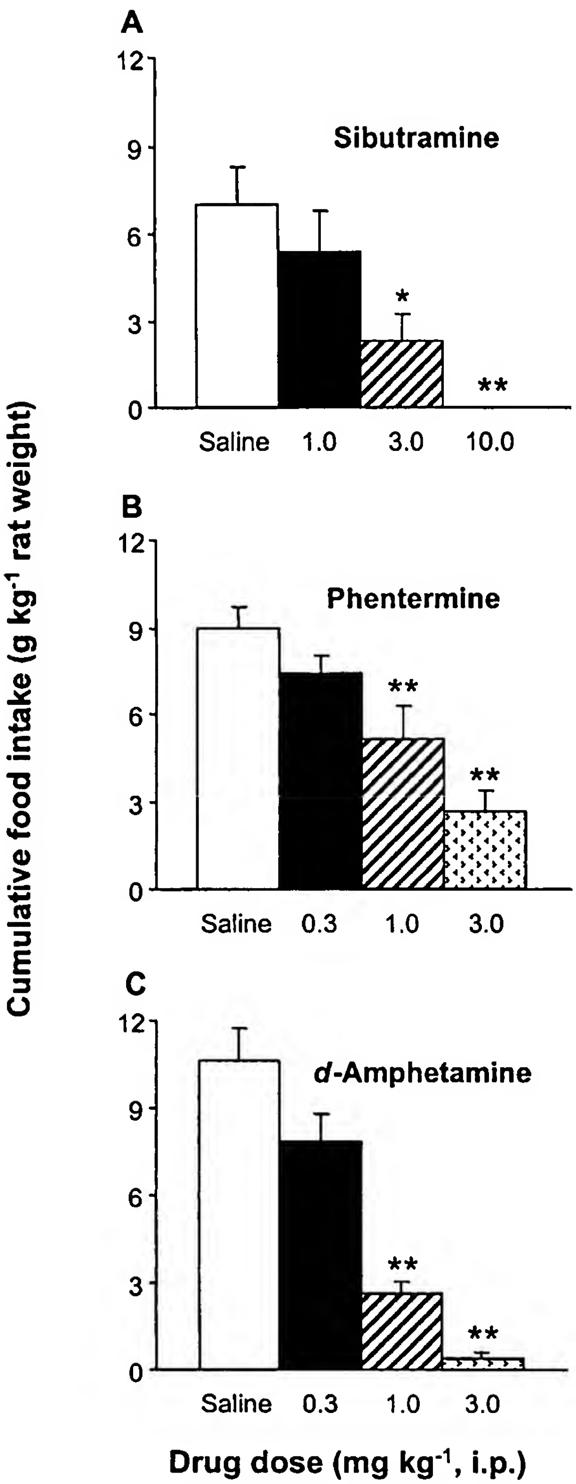

Fig. 1. Effects of (A) sibutramine, (B) phentermine and (C) *d*-amphetamine at 2 h on food intake in freely feeding rats. Results are expressed as treatment group mean ± SEM (n = 6–8). $^{*}P < 0.05$; $^{**}P < 0.01$ significantly different from saline-treated group.

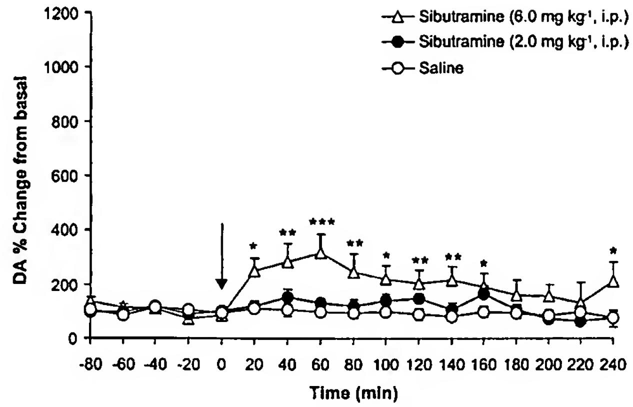

Fig. 2. Effects of treatment with sibutramine (2.0 and 6.0 mg kg^{-1}, i.p.), on extracellular DA in rat nucleus accumbens. Basal values were 0.69 ± 0.05 fmol 20 μl^{-1}. Drug or saline administration is indicated by the vertical arrow. Each data point represents mean ± SEM (n = 8–11). $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ significantly different from saline-treated group according to ANCOVA with post hoc *t*-test for multiple comparisons.

values are shown in Table II. In all microdialysis experiments, drugs were administered i.p. at a dose of 1× and 3× their 2 h ED_{50} value to inhibit food intake.

Effects of sibutramine on extracellular DA

At its 2 h ED_{50} to reduce food intake, sibutramine (2.0 mg kg^{-1}, i.p.) had no effect on extracellular DA levels in the nucleus accumbens of freely moving rats (Fig. 2). At the higher dose of 3× 2 h ED_{50}, sibutramine (6.0 mg kg^{-1}, i.p.) evoked a gradual increase in extracellular limbic DA, which reached a peak at 60 min post injection (+231 ± 87%, $P < 0.001$ compared to saline-treated controls) and which then plateaued and remained significantly elevated for ≤160 min (+94 ± 60%, $P < 0.05$ vs. saline-treated controls) with a further significant increase at 240 min (+184 ± 53%, $P < 0.05$ compared to saline-treated controls; Fig. 2). No behavioural changes were observed in rats treated with either dose of sibutramine compared to saline.

Effects of phentermine on extracellular DA

In contrast to sibutramine's lack of effect, phentermine, at its 2 h ED_{50} to reduce feeding (1.3 mg kg^{-1}, i.p.), produced a rapid increase in extracellular DA in the nucleus accumbens (Fig. 3) with a peak in levels at 20 min post treatment (+389 ± 247%, $P < 0.001$ compared to saline-treated controls). The increase in DA efflux was of relatively short duration, declining to saline-treated control values by 100 min. The effect of phentermine on DA efflux was more pronounced at 3× 2 h ED_{50} (3.9 mg kg^{-1}, i.p.). At this dose, phentermine produced a sharp increase in extracellular DA of +733 ± 358% ($P < 0.001$ vs. saline-treated controls) at 40 min (Fig. 3) which returned to control values at 160 min. At this higher dose of phentermine, rats displayed increased locomotor activity along with stereotypical

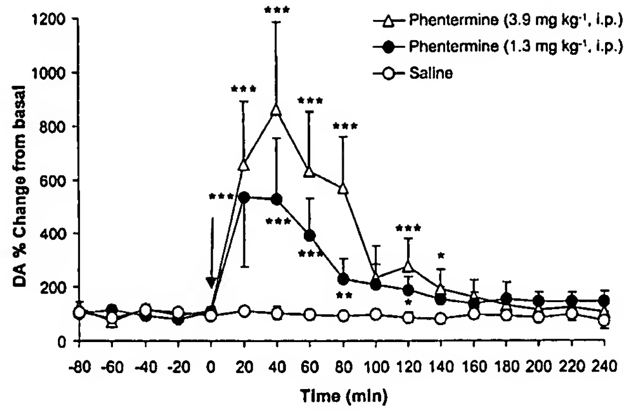

Fig. 3. Effects of treatment with phentermine (1.3 and 3.9 mg kg^{-1}, i.p.), on extracellular DA in rat nucleus accumbens. Basal values were 0.65 ± 0.08 fmol 20 μl^{-1}. Drug or saline administration is indicated by the vertical arrow. Each data point represents mean ± SEM (n = 8–11). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ significantly different from saline-treated group according to ANCOVA with post hoc t-test for multiple comparisons.

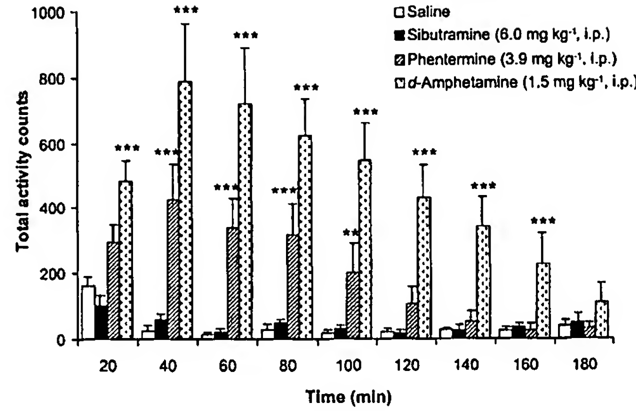

Fig. 5. Effects of sibutramine, phentermine, and d-amphetamine on rat locomotor activity. Activity is expressed as mean ± SEM (n = 8). **$P < 0.01$; ***$P < 0.001$ significantly different from saline-treated group according to two-way ANOVA with post-hoc least significant difference test.

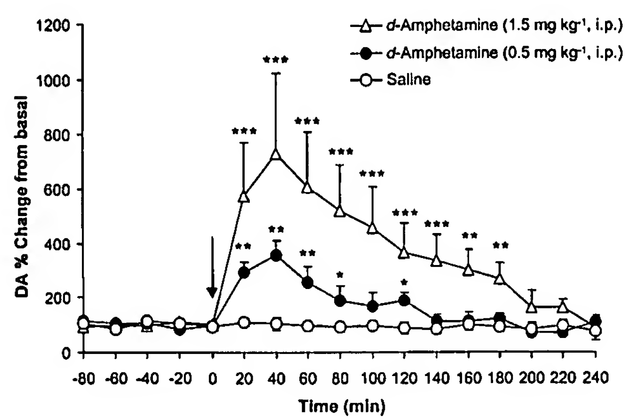

Fig. 4. Effects of treatment with d-amphetamine (0.5 and 1.5 mg kg^{-1}, i.p.), on extracellular DA in rat nucleus accumbens. Basal values were 0.81 ± 0.06 fmol 20 μl^{-1}. Drug or saline administration is indicated by the vertical arrow. Each data point represents mean ± SEM (n = 8–11). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ significantly different from saline-treated group according to ANCOVA with post hoc t-test for multiple comparisons.

behaviours, i.e., sniffing, gnawing, and excessive grooming.

The phentermine-induced elevation of extracellular DA was significantly greater at both doses compared to the rise in DA levels evoked by the corresponding dose of sibutramine over both 0–40 min ($P < 0.01$) and 40–80 min post treatment ($P = 0.01$).

Effects of *d*-amphetamine on extracellular DA

Analogous to the actions of phentermine, the 2 h ED_{50} dose of d-amphetamine (0.5 mg kg^{-1}, i.p.) rapidly increased limbic dialysate DA concentrations compared to saline treatment (Fig. 4). The increase was rapid in onset peaking at 40 min (+242 ± 89%, $P < 0.01$ vs. saline-treated controls) falling to control values at 100 min. d-Amphetamine (1.5 mg kg^{-1}, i.p.) evoked markedly greater DA efflux with a peak of +603 ± 319% ($P < 0.001$ compared to saline-treated controls) at 40 min post injection that declined gradually back to control values by 200 min (Fig. 4). The higher dose of d-amphetamine produced behavioural changes that were similar to those observed with the pharmacologically equivalent dose of phentermine.

The d-amphetamine-induced elevation of DA levels was significantly greater than the increases invoked by sibutramine at 0–40 min, 40–80 min ($P < 0.05$ both doses) and 80–120 min post-treatment ($P < 0.05$ for 3× ED_{50} dose only).

Locomotor activity

Sibutramine (6.0 mg kg^{-1}, i.p.) had no effect on rat locomotor activity (Fig. 5), whereas both phentermine (3.9 mg kg^{-1}, i.p.) and d-amphetamine (1.5 mg kg^{-1}, i.p.) significantly increased locomotor activity (+355% and +931%, respectively, compared to saline-treated controls; $P < 0.001$) over a 3-h post treatment period. A maximal increase in locomotor activity was observed at 40 min post-phentermine (+1530% compared to controls, $P < 0.001$), with activity remaining significantly elevated for up to 100 min post treatment (Fig. 5). d-Amphetamine also caused a maximum increase in locomotor activity at 40 min post treatment (+2915% compared to controls, $P < 0.001$; +85% compared to phentermine, $P < 0.01$) with rats displaying significantly elevated activity compared both to controls and to phentermine treatment for up to 160 min post treatment (Fig. 5).

Correlation between DA efflux and locomotor activity

A significant correlation was observed between the phentermine- and d-amphetamine-evoked increases in

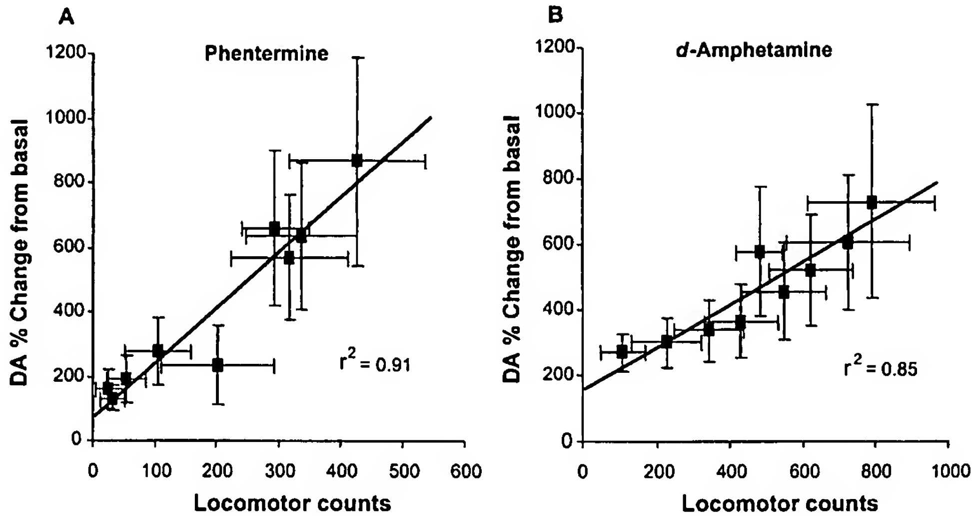

Fig. 6. Correlation between the increases in extracellular DA in the nucleus accumbens and locomotor activity in rats after administration of (A) phentermine (3.9 mg kg^{-1}, i.p.) and (B) *d*-amphetamine (1.5 mg kg^{-1}, i.p.).

locomotor activity and their elevation of DA efflux (Fig. 6). Linear regression analysis yielded r^2 values of 0.91 ($P < 0.001$, phentermine) and 0.85 ($P < 0.001$, *d*-amphetamine). There was no correlation between the effects of sibutramine on DA levels and locomotor activity ($r^2 = 0.01$, $P = 0.79$).

DISCUSSION

The effects of sibutramine on extracellular DA levels in the rat nucleus accumbens along with locomotor activity were compared to those of phentermine and *d*-amphetamine at pharmacologically equivalent doses in terms of inhibition of food intake.

All the drugs evaluated in this study, i.e., sibutramine, phentermine, and *d*-amphetamine, have been approved as treatments for obesity and are all believed to work through enhancement of central monoaminergic function. Sibutramine is a novel, arylcyclobutylalkylamine anti-obesity drug which produces its therapeutic effects via potent inhibition of NA and 5-HT uptake. In vivo, these effects are mediated almost exclusively in animals and man via its active metabolites, Metabolites 1 and 2 (Luscombe et al., 1989). Phentermine is a structural analogue of *d*-amphetamine that has been used as an anti-obesity agent in the USA for over 30 years. Phentermine's weight-loss action was initially ascribed to its sympathomimetic properties involving NA and DA (Samanin et al., 1975), but in later years the dopaminergic component of phentermine has been largely overlooked (Cerulli et al., 1998; Finer, 1997); this is despite recent in vivo evidence to show that phentermine does activate the dopaminergic system (Balcioglu and Wurtman, 1998; Shoaib et al., 1997). *d*-Amphetamine was one of the first drugs to be widely employed for management of obesity, but its use is no longer recommended due to the abuse potential of the drug (Cerulli et al., 1998). Like phentermine, *d*-amphetamine is a β-phenylethylamine and is an indirect adrenergic and dopaminergic agonist which stimulates catecholamine neurotransmission by evoking the release of NA and DA from nerve terminals, the latter effect being considered responsible for the abuse potential of the drug.

In this study, we determined the relative potency of these drugs as inhibitors of [^{3}H]DA uptake into synaptosomes prepared from rat nucleus accumbens. The results obtained with sibutramine and Metabolites 1 and 2 agree closely with earlier findings obtained using rat striatal synaptosomes (Cheetham et al., 1990). Thus, sibutramine is an extremely weak [^{3}H]DA uptake inhibitor in vitro, whereas Metabolites 1 and 2 are relatively potent with K_i values of 17 and 26 nM, respectively. *d*-Amphetamine is a moderately potent DA uptake inhibitor in vitro, while phentermine, like sibutramine, is extremely weak. Turning to effects on DA release, Heal et al. (1992) have shown that neither sibutramine nor Metabolites 1 and 2 release [^{3}H]DA from rat striatal slices at concentrations 10^{-7}–10^{-5} M, which are ~400-fold greater than their K_i values for the inhibition of [^{3}H]DA uptake in vitro. In contrast, *d*-amphetamine significantly releases [^{3}H]DA from rat striatal slices at concentrations as low as 10^{-7} M (Heal et al., 1996); therefore, there is no separation between the concentration at which this drug releases or blocks the uptake of [^{3}H]DA in vitro. Analogous to its weak effect on DA uptake in vitro, phentermine does not cause significant release of this monoamine until 10^{-5} M (Lancashire et al., 1998).

Sibutramine, phentermine, and *d*-amphetamine all produced potent dose-dependent inhibition of food intake in rats with 2 h ED_{50} values in the low mg kg^{-1} i.p. range. In subsequent microdialysis experiments, we looked at the effects of these drugs at a dose which profoundly inhibits feeding, i.e., the ED_{50} to reduce food intake at 2 h, and also 3× 2 h ED_{50} values which would almost totally abolish this response. At the lower

dose, sibutramine did not elevate extracellular DA concentrations in the nucleus accumbens of freely moving rats. In contrast, the pharmacologically equivalent dose of phentermine and *d*-amphetamine caused marked increases in DA efflux. In both instances, the increase in extracellular DA was rapid in onset and of short duration. In view of phentermine's weak actions in vitro, it may appear surprising that phentermine and *d*-amphetamine should show equal potency to enhance extracellular DA concentrations in vivo. However, previous experience has shown that data from in vitro experiments can markedly underestimate the impact that releasing agents exert on monoaminergic function in vivo (Heal et al., 1998b; Lancashire et al., 1998; Prow et al., 1999).

These microdialysis results are consistent with data obtained from an analysis of the actions of sibutramine, phentermine, and *d*-amphetamine on feeding behaviour. Feeding is terminated by satiety and, in rats, this is associated with a specific repertoire of behaviours described by Antin et al. (1975) as the "satiety sequence"; a key element of this is postprandial resting. Drugs such as sibutramine and *d*-fenfluramine accelerate but do not alter this physiological response (Halford et al., 1995). However, the reduction in food intake with phentermine is associated with disruption of the satiety sequence (Jackson, unpublished observations) and with *d*-amphetamine occurs only at psychostimulant doses characterised by stereotyped behaviours and increased activity (Halford et al., 1995).

The hypothesis that sibutramine reduces food intake and increases energy expenditure via NA and 5-HT uptake inhibition (Connoley et al., 1999; Jackson et al., 1997a,b) was also examined using the technique of in vivo microdialysis in freely moving rats. At doses similar to its 2 h ED_{50} to reduce food intake, sibutramine has been shown to significantly elevate extracellular NA levels both in the frontal cortex and hypothalamus (Wortley et al., 1999a,b). Similarly, at 3 mg kg^{-1}, i.p., increases in hypothalamic extracellular 5-HT concentrations were observed after administration of sibutramine or Metabolite 1, although they only reached statistical significance in the case of the metabolite at this dose (Gundlah et al., 1997).

In the present study, at 3× its 2 h ED_{50} to reduce feeding, sibutramine evoked a moderate increase in limbic, extracellular DA concentrations (maximum increase = +231%). The in vivo effects of sibutramine on DA levels have been the subject of preliminary studies in the striatum and hypothalamus (Balcioglu et al., 1998) with the highest dose of sibutramine (10 mg kg^{-1}, i.p.) resulting in increases in DA in both brain regions that were similar to those reported here using the dose of 6 mg kg^{-1}, i.p. As expected, at the higher dose phentermine and *d*-amphetamine markedly enhanced DA efflux in the nucleus accumbens, with peak increases of +733% and +603%, respectively. The phentermine-evoked release of extracellular DA observed in the present study is of a similar magnitude and duration to those observed in previous studies in rat nucleus accumbens (Shoaib et al., 1997) and striatum (Balcioglu and Wurtman, 1998). Similarly, our findings with *d*-amphetamine are consistent with those showing that this compound potently increases extracellular DA in the nucleus accumbens of freely moving rats (Di Chiara et al., 1993).

Although relative increases in extracellular neurotransmitter levels measured by microdialysis are important, they provide few clues as to the functional consequences of such changes in vivo. To obtain a handle on this latter issue, locomotor activity was measured in parallel groups of rats as an index of enhanced limbic dopaminergic function and psychostimulant action (Jackson et al., 1975; Kelly et al., 1975). In this part of the investigation, the drugs were examined at 3× their respective 2 h ED_{50} doses to inhibit feeding, where each produced significant increases in extraneuronal DA concentrations in the nucleus accumbens. At this dose, sibutramine did not evoke either locomotion or stereotypy. In contrast, phentermine and *d*-amphetamine markedly increased locomotor activity consistent with enhanced dopaminergic function. The phentermine- and *d*-amphetamine-evoked elevation of DA efflux were coincident with an increase in locomotor activity, the time-course of which significantly correlated with that of DA release. By observing the time-courses of both the phentermine- and *d*-amphetamine-induced increases in 1) extracellular DA efflux from the nucleus accumbens and 2) locomotor activity, it is clear that the increase in locomotor activity ceases when the elevated DA levels in the nucleus accumbens fall to below 300% of baseline values. The lack of effect on locomotor activity by sibutramine is coincident with the fact that this drug did not increase DA efflux above this apparent threshold for a prolonged period.

While there is a close correlation between the time-course of the increases in DA efflux and locomotor activity, there appears to be a disparity in the maximum effects of these changes. The increases in DA evoked by both phentermine and *d*-amphetamine in the nucleus accumbens were of a similar magnitude. However, the *d*-amphetamine-induced increase in locomotor activity was significantly greater ($P < 0.001$) compared to that observed in phentermine-treated animals. An increase in locomotor activity is believed to arise primarily from stimulation of dopaminergic transmission in the nucleus accumbens, but there is also evidence to support a weaker contribution from both serotonergic (Callaway et al., 1990) and noradrenergic neurones (Darracq et al., 1998). In vitro release studies have demonstrated that *d*-amphetamine (10 μM) causes the release of 5-HT from rat striatal slices, whereas the same concentration of phentermine does not (Lancashire et al., 1998). This effect is mirrored in

vivo in both the nucleus accumbens (Kankaanpää et al., 1998; Shoaib et al., 1997) and striatum (Balcioglu and Wurtman, 1998). Similarly, both *d*-amphetamine and phentermine increase NA levels in the rat hypothalamus in vivo (Viggers et al., 1999; Wortley et al., 1999a). Thus, the combined effect of *d*-amphetamine on DA, 5-HT, and NA efflux in the nucleus accumbens and/or other brain regions may account for the more pronounced effect of *d*-amphetamine on rat locomotor activity compared to phentermine, given that the *d*-amphetamine- and phentermine-induced increases in DA efflux were of a similar magnitude.

In conclusion, at a dose which profoundly inhibits feeding in rats sibutramine has no effect on extracellular DA levels in the rat nucleus accumbens, the area most likely to be involved in the rewarding and reinforcing properties of drugs of abuse. At the functionally equivalent dose, both phentermine and *d*-amphetamine caused marked rises in DA efflux, the effect of phentermine being of at least the same magnitude as that seen with *d*-amphetamine. At 3× its ED_{50} value, sibutramine resulted in a moderate increase in DA efflux, but the elevation was not of sufficient magnitude nor duration to have any effect on rat locomotor activity, unlike the rise in DA levels evoked by phentermine and *d*-amphetamine at functionally equivalent doses. Thus, no evidence was found for the involvement of DA release in the reduction of food intake by sibutramine. Furthermore, these findings are consistent with the recent report that sibutramine lacks psychostimulant abuse potential in humans (Cole et al., 1998).

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NPY5R Antagonism Does Not Augment the Weight Loss Efficacy of Orlistat or Sibutramine

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Abstract

Introduction

Methods

Results

Summary

Figure 1. Patient disposition. AE, adverse event.

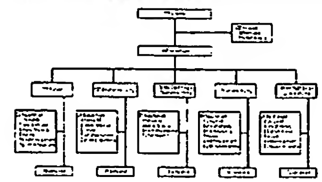

Figure 2. Mean change from baseline in body weight (kg) over 24 weeks of treatment using last observation carried forward (Modified Intention to Treat Population). Least Squares (LS) Mean estimates (84% Confidence Interval) based on ANCOVA model with terms for treatment, baseline body weight, center, and run-in weight change.

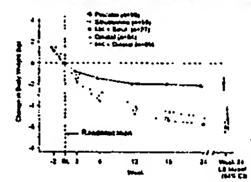

Figure 3. Change from baseline in systolic blood pressure (mmHg) over 24 weeks of treatment (Modified Intention to Treat Population). Observed data plotted from Week -2 to 24. Sample size corresponds to Week 24. 84% Confidence Interval shown on Week 24.

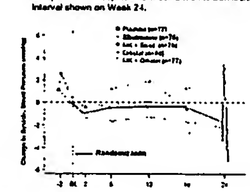

Figure 4. Cumulative dropout rate over time by treatment group.

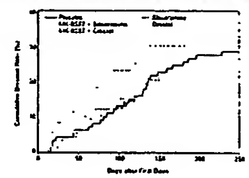

Figure 5. Time to first event per patient, expressed as cumulative incidence rate, of gastrointestinal adverse experiences by treatment group for the Modified Intention to Treat Population.

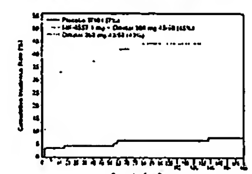

Table 1. Patient baseline characteristics for all randomized patients.

Table 2. Selected metabolic and CV outcomes for MITT population.

Table 3. Clinical Adverse Experience Summary.

References

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NPY5R Antagonism Does Not Augmen

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Abstract

Background: Currently approved drugs for weight control, sibutramine and orlistat, have limited efficacy, which may be related to counter-regulatory mechanisms including the orexigenic neuropeptide Y (NPY) pathway. The objective of this study was to evaluate whether MK-0557, a highly selective NPY Y5 receptor (NPY5R) antagonist, potentiates the weight loss effects of sibutramine and orlistat.

Research Methods and Procedures: We conducted a double-blind, placebo-controlled study which randomized 497 obese patients (BMI 30-43 kg/m^2) to 1 of 5 treatment arms [placebo (n=101); sibutramine 10 mg q.d. (n=100); MK-0557 1 mg q.d. plus sibutramine 10 mg q.d. (n=98); orlistat 120 mg t.i.d. (n=99); MK-0557 1 mg q.d. plus orlistat 120 mg t.i.d. (n=99)] in conjunction with a hypocaloric diet and exercise.

Results: In the Modified Intention to Treat population, imputing missing data using Last Observation Carried Forward, the least squares (LS) mean difference (95% CI) between MK-0557 + sibutramine and sibutramine alone was 0.1 (-1.6, 1.4) kg (p=0.892) and between MK-0557 + orlistat and orlistat alone was 0.9 (-2.4, 0.6) kg (p=0 250). Sibutramine alone induced an LS mean weight loss of -5.9 (-6.9, -4.9) kg versus -4.6 (-5.7, -3.6) kg for orlistat. Seventy-one percent in the placebo, 76% in the sibutramine alone, 80% in the MK-0557 + sibutramine, 69% in the orlistat alone, and 76% in the MK-0557 + orlistat groups completed the study.

Conclusions: In this study, blockade of the NPY5R with MK-0557 did not increase the weight loss efficacy of either orlistat or sibutramine. Sibutramine was associated with greater weight loss and better patient retention than orlistat, although the differences between the two drugs were not statistically significant.

Introduction

Two presently approved medications for weight loss treatment are orlistat and sibutramine. Orlistat is an inhibitor of gastrointestinal and pancreatic lipases that promotes weight loss and negative energy balance through reducing fat absorption (1, 2). Sibutramine is a selective inhibitor of the reuptake of norepinephrine and serotonin and, to a lesser extent, dopamine, which facilitates weight loss through both suppression of food intake and augmentation of energy expenditure (3, 4).

While the orlistat and sibutramine development programs were initiated over two decades ago, new novel targets are beginning to be critically evaluated with translational research. In this respect, neuropeptide Y (NPY) has been characterized as a potent orexigenic factor that is a key component of an anabolic network that promotes food intake and decreases energy expenditure (5-9). MK-0557 is a highly selective NPY5R antagonist that induced modest, dose-dependent weight loss in a 12-week proof-of-concept clinical trial in obese patients (10). This dose-ranging study combined with receptor occupancy data from positron emission tomography (PET, 10) established 1 mg as the appropriate daily dose of MK-0557 for clinical studies.

As part of our evaluation of MK-0557 as a clinical candidate we examined the weight-loss effects of this NPY5R antagonist when co-administered with orlistat and sibutramine. Our experimental protocol also provided the opportunity for a head-to-head comparison of orlistat and sibutramine.

Methods

Hypotheses and Study Design

Primary hypotheses: MK-0557 1 mg q.d. co-administered with (1) sibutramine 10 mg q.d. for 24 weeks reduces body weight more than sibutramine alone; (2) orlistat 120 mg t.i.d. for 24 weeks reduces body weight more than orlistat alone; and (3) sibutramine or orlistat for 24 weeks is safe and well tolerated.

The hypothesis was examined in a multicenter, double blind, randomized, placebo-controlled study. Prior to randomization, there was a 2-week diet/exercise and single blind placebo run-in period. Patients were instructed to follow a diet 500 kcal/day below their weight maintenance requirements, based upon an estimation of energy expenditure (11).

Eligible patients were randomized equally (Figure 1) to each of 5 treatment arms (placebo; sibutramine 10 mg q.d.; MK-0557 1 mg q.d. plus sibutramine 10 mg q.d.; orlistat 120 mg t.i.d.; MK-0557 1 mg q.d. plus orlistat 120 mg t.i.d.) and continued diet/exercise counseling. The primary measure of efficacy was change from baseline in body weight.

Obese patients with BMI between 30 kg/m^2 and 43 kg/m^2, between the ages of 18 and 65 years, inclusive, and who met other entry criteria were eligible to participate.

Statistical Analysis

The primary analysis population was a modified intention to treat (MITT) population, which was composed of subjects who received at least one dose of randomized study medication and had at least one post-randomization/baseline weight measurement. For evaluation of change from baseline, patients who had both a baseline and at least one post-baseline measurement were included in the analysis. Missing data were imputed using the last observation carried forward (LOCF). A repeated measures ANCOVA was also used to analyze the observed (i.e., without LOCF imputation); results were consistent with the LOCF analysis and are thus not reported here.

The efficacy hypotheses were evaluated by comparing the mean change from baseline in body weight using an analysis of covariance (ANCOVA) model with terms for weight loss during the run-in, baseline body weight, treatment, and center.

This study was powered to detect a 2.3 (2.0) kg difference with 90% (80%) power, assuming a standard deviation of 4.78 kg, level 0.05 for the primary hypotheses and 90 patients per treatment arm. The expected half-width of the 95% confidence interval was 1.4 kg.

Results

Patients

Patient characteristics are summarized in Table 1. Overall, the study consisted mainly of white (~75-83%) women (~80-85%) who were moderately obese with a baseline BMI of ~35 kg/m^2

Patient disposition is outlined in Figure 1. A total of 719 patients were screened and from these 497 patients were randomized to placebo (n=101), sibutramine (n=100), MK-0557 + sibutramine (n=98), orlistat (n=99), and MK-0557 + orlistat (n=99). At completion of the protocol, 73% (n=368) of the 497 patients remained in the study, 71% (n=72) in the placebo group, 76% (n=76) in the sibutramine group, 79% (n=77) in the MK-0557 + sibutramine group, 69% (n=68) in the orlistat group, and 76% (n=75) in the MK-0557 + orlistat group.

MK-0557 + sibutramine versus sibutramine alone

After 24 weeks of treatment, MK-0557 did not induce significant weight loss when co-administered with sibutramine compared to sibutramine alone (p=0.892) (Table 2 and Figure 2). In the MITT population, the least squares (LS) mean difference (95% CI) between MK-0557 + sibutramine and sibutramine alone was -0.1 (-1.6, 1.4) kg. No significant differences were observed in the per-protocol population or in the 5% and 10% responder analyses.

MK-0557 + orlistat versus orlistat

After 24 weeks of treatment, MK-0557 did not induce significant weight loss when coadministered with orlistat compared to orlistat alone (p=0.250) (Table 2 and Figure 2). In the MITT population, the LS mean difference (95% CI) between MK-0557 + orlistat and orlistat alone was -0.9 (-2.4, 0.6) kg. No significant differences were observed in the per-protocol population or in the 5% and 10% responder analyses.

Sibutramine versus orlistat

The least squares (LS) mean change in body weight (95% CI) was -5.9 (-6.9, -4.9) kg in the sibutramine group and -4.6 (-5.7, -3.6) kg in the orlistat group, as compared to a mean change of -1.8 (-2.9, -0.8) kg for placebo (Figure 2 and Table 2). Both sibutramine and orlistat induced statistically significant changes in body weight (p<0.001 for both compounds vs. placebo), and the difference between the two compounds approached significance (p=0.097). No significant differences between sibutramine and orlistat were observed in the per-protocol population or in the 5% and 10% responder analyses

Clinical evaluations and adverse events

Systolic (and diastolic) blood pressure was almost unchanged over 24 weeks in the placebo group (Table 2). Orlistat treatment was accompanied by a small reduction in both systolic and diastolic blood pressure (-1.4 and -1.2 mmHg, respectively), while sibutramine treatment was accompanied by a 2.1 mmHg elevation in systolic blood pressure. The systolic blood pressure difference for orlistat and sibutramine (3.6 mmHg; 95% CI: 0.9, 6.1 mmHg) was significant (p=0.008) while no significant treatment difference was observed in diastolic blood pressure. The temporal relations in systolic blood pressure changes over the study period are shown from the per-protocol population in Figure 3

There were no deaths or serious drug-related adverse events (Table 3). The highest proportions of patients with drug-related adverse experiences were in the orlistat groups (40.4% for orlistat alone and 51.5% for orlistat + MK-0557) followed by the sibutramine (28.0% for sibutramine alone and 31.6% for sibutramine + MK-0557) and placebo (17.8%) groups. Of the patients discontinuing due to a drug-related adverse experience, 4% were in the orlistat alone and 1% in both the sibutramine alone and placebo groups. The study dropout rate (Figure 4) was highest in the orlistat alone group (31%) and lowest in the Sibutramine + MK-0557group (21%).

The most common reported clinical adverse experiences for the sibutramine group were dry mouth (6% vs. 1% in placebo and 1% in orlistat groups) and constipation (11% vs. 4% in placebo and 2% in orlistat groups). Diarrhea (17.2% vs. 3% in placebo and 5% in sibutramine groups), loose stools (8.1% vs. 2% in placebo and 1% in sibutramine groups), and other related gastrointestinal effects were the most common adverse events in the orlistat group. The orlistat-related gastrointestinal events tended to occur within the first four-weeks of treatment (Figure 5).

Summary

Our main observation is that in a randomized controlled clinical trial NPY5R antagonism with MK-0557 did not lead to additional weight loss beyond that observed with either sibutramine or orlistat alone. The mechanism(s) leading to lack of additivity are unknown but include statistical and biological hypotheses.

The current investigation is one of the first randomized, double-blind studies that include a head-to-head comparison of orlistat and sibutramine. In the present 24-week study, orlistat alone or in combination with MK-0557 led to significant weight loss compared to placebo of 2.8 and 3.7 kg, respectively. The respective weight loss above placebo at 24 weeks observed in our study for sibutramine with or without MK-0557, 4.2 kg and 4.0 kg, was greater than that with orlistat, although the differences were not statistically significant.

Our findings and those other head-to-head comparison studies suggest the overall weight loss efficacy of the two drugs is similar, with a small numerical advantage for sibutramine. However, the two drugs were not equally well tolerated. In our study, dry mouth and constipation were the two most frequently reported adverse events with sibutramine and retention of patients in the two sibutramine groups was similar to that of the placebo group. In contrast, gastrointestinal adverse events were pervasive and most likely accounted for the relatively high early dropout rate in both orlistat groups. Blood pressure, as expected, declined modestly (~1-2 mmHg) with orlistat treatment and increased to about the same extent in the sibutramine-treated patients.

In summary, our study demonstrated that the co-administration of a selective NPY5R antagonist with either of two conventional weight loss therapies, orlistat or sibutramine, did not result in a statistically significant

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Figure 1. Patient disposition. AE, adverse event.

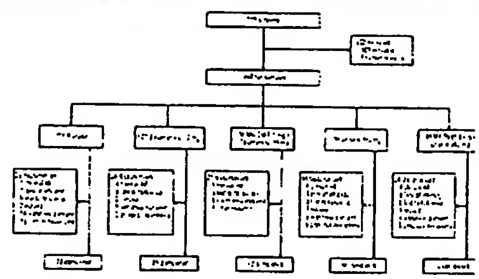


Figure 2. Mean change from baseline in body weight (kg) over 24 weeks of treatment using last observation carried forward (Modified Intention to Treat Population)
†Least Squares (LS) Mean estimates (84% Confidence Interval) based on ANCOVA model with terms for treatment, baseline body weight, center, and run-in weight change.

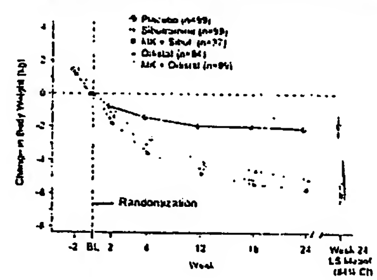
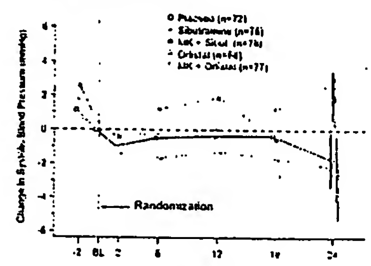


Figure 3. Change from baseline in systolic blood pressure (mmHg) over 24 weeks of treatment (Modified Intention to Treat Population). Observed data plotted from Week -2 to 24. Sample size corresponds to Week 24. 84% Confidence Interval shown on Week 24.



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icacy of Orlistat or Sibutramine

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Figure 4. Cumulative dropout rate over time by treatment group.

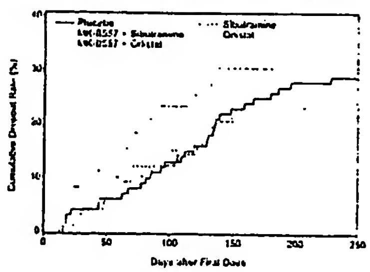

Figure 5. Time to first event per patient, expressed as cumulative incidence rate, of gastrointestinal adverse experiences by treatment group for the Modified Intention to Treat Population.

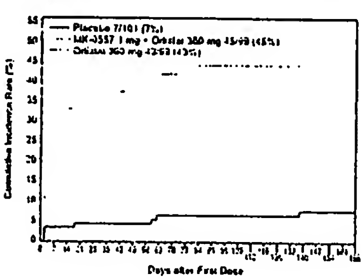

Table 1. Patient baseline characteristics for all randomized patients.

	Placebo (n=101)	Sibutramine (n=100)	MK-0557 + Sibutramine (n=98)	Orlistat (n=99)	MK-0557 + Orlistat (n=99)
Race (white)*	84 (83.2%)	77 (77.0%)	79 (80.6%)	78 (78.8%)	75 (75.8%)
Gender (women)*	83 (82.2%)	85 (85.0%)	79 (80.6%)	83 (83.8%)	79 (79.8%)
Age (years)[1]	42.6 (10.8)	40.9 (11.1)	40.7 (9.3)	41.9 (9.4)	42.4 (10.5)
BMI (kg/m²)[1]	35.9 (3.5)	35.8 (3.8)	35.3 (3.4)	35.3 (3.6)	35.7 (3.6)
Weight (kg)[1]	97.3 (15.2)	98.0 (15.4)	95.1 (13.5)	96.3 (12.3)	97.1 (14.0)

*Data are number (%). [1]Data are mean (SD)

Table 2. Selected metabolic and CV outcomes for MITT population.

	Baseline	Week 24	LS change
Weight (kg)			
Placebo	97.3 (15.2)	95.2 (16.1)	-1.8 (-2.9, -0.8)
Sibutramine	98.0 (15.4)	92.2 (15.8)	-5.9 (-6.9, -4.9)
MK-0557 + Sibutramine	96.1 (13.5)	90.4 (14.7)	-6.0 (-7.1, -4.9)
Orlistat	96.3 (12.3)	91.2 (12.7)	-4.6 (-5.7, -3.6)
MK-0557 + Orlistat	97.1 (14.0)	91.5 (14.8)	-5.5 (-6.6, -4.5)
Systolic blood pressure (mmHg)			
Placebo	117.7 (11.2)	117.8 (12.9)	0.1 (-1.7, 1.9)
Sibutramine	117.3 (11.0)	119.6 (10.9)	2.1 (0.3, 3.8)
MK-0557 + Sibutramine	115.4 (11.5)	117.5 (12.2)	1.2 (-0.6, 3.1)
Orlistat	116.6 (10.7)	115.5 (11.6)	-1.4 (-3.2, 0.5)
MK-0557 + Orlistat	118.1 (11.0)	115.1 (11.4)	-2.8 (-4.7, -1.0)
HDL Cholesterol (mg/dL)			
Placebo	49.3 (9.2)	49.5 (10.0)	1.0 (-1.7, 3.8)
Sibutramine	50.1 (12.9)	51.3 (12.0)	3.5 (0.8, 6.2)
MK-0557 + Sibutramine	49.2 (12.8)	51.0 (12.9)	6.2 (3.4, 8.9)
Orlistat	49.5 (11.9)	49.3 (10.9)	0.5 (-2.3, 3.4)
MK-0557 + Orlistat	49.9 (11.4)	49.3 (12.7)	1.1 (-1.7, 3.9)
Triglycerides (mg/dL)			
Placebo	109.5 (53.0)	119.0 (69.8)	3.3 (-5.8, 12.4)
Sibutramine	115.5 (73.5)	108.5 (70.7)	-9.0 (-16.1, -1.8)
MK-0557 + Sibutramine	119.0 (87.4)	101.0 (68.8)	-6.0 (-14.7, 2.7)
Orlistat	129.0 (75.3)	126.0 (56.6)	-2.2 (-9.6, 5.3)
MK-0557 + Orlistat	112.0 (47.4)	111.0 (78.1)	1.5 (-9.3, 12.3)
LDL Cholesterol (mg/dL)			
Placebo	111.9 (30.9)	116.0 (30.6)	7.8 (3.9, 11.6)
Sibutramine	115.4 (27.7)	120.2 (28.8)	5.5 (1.7, 9.3)
MK-0557 + Sibutramine	114.1 (25.7)	117.6 (32.6)	2.5 (-1.4, 6.3)
Orlistat	114.5 (27.8)	114.0 (29.1)	0.5 (-3.5, 4.5)
MK-0557 + Orlistat	113.8 (29.0)	107.4 (31.5)	-4.6 (-8.5, -0.7)

Data at Baseline and Week 24 are observed mean (SD) except for triglycerides (TG) that are median (SD). Data for change from baseline are LS mean change (95% CI) except for triglycerides (TG) that are median change (95% CI).

Table 3. Clinical Adverse Experience Summary.

	Placebo (N = 101)		Sibutramine 10 mg (N = 100)		MK-0557 1 mg + Sibutramine 10 mg (N = 98)		Orlistat 360 mg (N = 99)		MK-0557 1 mg + Orlistat 360 mg (N = 99)	
Number (%) of patients:	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
With one or more adverse experiences	68	(67.3)	68	(68.0)	64	(65.3)	69	(69.7)	84	(84.8)
With no adverse experience	33	(32.7)	32	(32.0)	34	(34.7)	30	(30.3)	15	(15.2)
With drug-related adverse experiences[1]	18	(17.8)	28	(28.0)	31	(31.6)	40	(40.4)	51	(51.5)
With serious adverse experiences	5	(5.0)	6	(6.0)	2	(2.0)	2	(2.0)	2	(2.0)
With serious drug-related adverse experiences	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
Who died	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
Discontinued due to adverse experiences	1	(1.0)	4	(4.0)	6	(6.1)	6	(6.1)	7	(7.1)
Discontinued due to drug-related adverse experiences	1	(1.0)	1	(1.0)	3	(3.1)	4	(4.0)	5	(5.1)
Discontinued due to serious adverse experiences	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
Discontinued due to serious drug-related adverse experiences	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)

[1]Determined by the investigator to be possible, probably or definitely drug related

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Effects of Sibutramine Plus Orlistat in Obese Women Following 1 Year of Treatment by Sibutramine Alone: A Placebo-Controlled Trial

Thomas A. Wadden, Robert I. Berkowitz, Leslie G. Womble, David B. Sarwer, Marjorie E. Arnold, and Carrie M. Steinberg

Abstract

WADDEN, THOMAS A., ROBERT I. BERKOWITZ, LESLIE G. WOMBLE, DAVID B. SARWER, MARJORIE E. ARNOLD, AND CARRIE M. STEINBERG. Effects of sibutramine plus orlistat in obese women following 1 year of treatment by sibutramine alone: a placebo-controlled trial. *Obes Res.* 2000;8:431–437.

Objective: This study assessed whether adding orlistat to sibutramine would induce further weight loss in patients who previously had lost weight while taking sibutramine alone.

Research Methods and Procedures: Patients were 34 women with a mean age of 44.1 ± 10.4 years, weight of 89.4 ± 13.8 kg, and body mass index (BMI) of 33.9 ± 4.9 kg/m^2 who had lost an average of 11.6 ± 9.2% of initial weight during the prior 1 year of treatment by sibutramine combined with lifestyle modification. Patients were randomly assigned, in double-blind fashion, to sibutramine plus orlistat or sibutramine plus placebo. In addition to medication, participants were provided five brief lifestyle modification visits during the 16-week continuation trial.

Results: Mean body weight did not change significantly in either treatment condition during the 16 weeks. The addition of orlistat to sibutramine did not induce further weight loss as compared with treatment by sibutramine alone (mean changes = +0.1 ± 4.1 kg vs. +0.5 ± 2.1 kg, respectively).

Discussion: These results must be interpreted with caution because of the study's small sample size. The findings, however, suggest that the combination of sibutramine and orlistat is unlikely to have additive effects that will yield mean losses ≥15% of initial weight, as desired by many obese individuals.

Key words: sibutramine, orlistat, obesity, women, weight loss

Introduction

Two medications, sibutramine (Meridia; Knoll Pharmaceutical Co., Mt. Olive, NJ) (1) and orlistat (Xenical; Roche Laboratories, Nutley, NJ) (2), are currently approved by the Food and Drug Administration for weight loss and the maintenance of weight loss. Sibutramine is a combined norepinephrine-serotonin re-uptake inhibitor, whereas orlistat is a gastric and pancreatic lipase inhibitor. In controlled trials, sibutramine (15 mg once a day) was associated at 1 year with a 7% reduction in initial weight (1,3) and orlistat (120 mg, three times a day [TID]) with a 10% reduction (2,4–6). In both cases, the difference in weight loss between the medication and placebo conditions (i.e., placebo-subtracted weight loss) was approximately 4% to 5%.

Obese individuals want to lose two to three times more weight than is typically possible with current medications (7). Several investigators have suggested that larger weight losses might be achieved by combining weight loss agents (8–10). The present pilot study explored the benefits of adding orlistat to sibutramine in obese women who had lost an average of 11.6 ± 9.2% of their initial weight during 1 year of treatment by sibutramine alone. All women in the pilot study continued to receive sibutramine for 16 weeks; in addition, half of them were randomly assigned to orlistat and the other half to placebo. These two medications would appear to be excellent candidates for combined therapy because of their different mechanisms of action.

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Research Methods and Procedures

Patients

Patients were 34 volunteers from a group of 43 women who had completed a 1-year treatment program that combined sibutramine (10 to 15 mg/d) with different amounts of lifestyle modification. As described in a separate report (11), the 43 participants lost an average of 12.0 ± 9.6 kg at 1 year, but there were marked differences among patients based on the program of lifestyle modification they received.

The 34 volunteers in the continuation study were told that all participants would receive sibutramine for an additional 16 weeks and that half of them also would be assigned at random (in double-blind fashion) to orlistat and the other half to placebo. The stated goal of the study was to determine whether the addition of orlistat would be associated with greater weight loss (or better maintenance of weight loss) than would continued treatment by sibutramine alone. Patients gave their informed consent to participate in the continuation study, which was approved by the University of Pennsylvania's Committee on Studies Involving Human Beings. Patients' characteristics, before randomization, are shown in Table 1. ANOVA showed that patients in the two conditions did not differ significantly on any of the baseline measures, including weight loss during the prior 1-year program.

Procedures

At baseline (i.e., week 52), all patients met with a physician (R. I. B.) who examined their health and told them to continue to take sibutramine (10 to 15 mg once a day) in the morning. In addition, they were instructed to take one capsule of the investigational medication within ± 1 hour of lunch, dinner, and an evening snack. We decided not to prescribe orlistat in the morning because 14 of 34 (41%) patients indicated that they ate breakfast infrequently (i.e., 0 to 3 times a week). Of the 20 remaining participants, 12 (i.e., 35% of the total sample) reported that they usually ate a breakfast that was determined to contain ≤10 g of fat. For most women, evening snacking appeared to present a greater risk for overeating than did breakfast.

Patients were instructed to limit their fat intake to a maximum of 20 g per meal (or snack), and 60 g per day, to minimize possible gastrointestinal events, including oily stools, oily spotting, fecal urgency, and related side effects (2,4–6). They were warned that they would not be able to predict the temporal occurrence of such events. Participants were instructed to take the medication three times a day, at the designated times, even if they missed a meal or snack. This was done to facilitate their taking the medication as regularly as possible. Patients were also instructed to take a multivitamin supplement every morning to prevent possible decreases in levels of fat-soluble vitamins.

At week 53, patients returned to see the physician who assessed their response to both sibutramine and the experimental medication. Follow-up medical visits were scheduled at weeks 56 and 68 (or more frequently, as needed).

Lifestyle Modification. All patients met with a registered dietitian or doctoral-level psychologist for 30 minutes at weeks 52, 56, 60, 64, and 68. At the first visit, patients' energy requirements were calculated, and they were instructed to consume a diet of 1200 to 1600 kcal/d, representing a deficit of approximately 600 to 850 kcal/d. Patients were told to consume a balanced diet (of their choosing) with approximately 20% of calories from protein, 50% from carbohydrate, and ≤30% from fat. They were provided handouts on topics that included the Food Guide Pyramid, food labels, low-fat cooking, and meal planning. Each month the practitioner reviewed patients' food diaries and medication compliance. Participants also set monthly activity goals with an eventual objective of exercising five times a week for 30 to 40 minutes per bout.

Dependent Measures. Weight was measured at each visit with patients dressed in light clothing and without shoes. At week 68 (i.e., end of study), participants indicated whether they believed they had been assigned to orlistat or placebo. In addition, they completed a symptom inventory that assessed, for the prior week, the number of days that they had experienced various gastrointestinal events.

Attrition and Statistical Analyses

Three patients treated by sibutramine plus orlistat (i.e., combined therapy) and five treated by sibutramine alone discontinued treatment prematurely. Table 2 summarizes the reasons for attrition and patients' weight loss at the time. A chi square test revealed no significant differences in dropout between conditions. Differences in weight loss between conditions during the 16-week trial were compared using analysis of covariance, with weight loss at the end of the first year of treatment (by sibutramine alone) taken as the covariate. Data were analyzed using both an end-point analysis (which included only treatment completers) and a last-observation-carried-forward analysis. The two sets of analyses reached the same statistical conclusions.

Results

Weight Loss

Figure 1 shows that body weight was essentially unchanged in both conditions during the 16-week continuation trial. ANOVA revealed neither an effect of time nor treatment condition. Thus, contrary to our hypothesis, the addition of orlistat to sibutramine did not significantly increase weight loss (or improve the maintenance of weight loss) as compared with the continued use of sibutramine alone (see Table 3).

A second ANOVA examined the effect of prior 1-year weight loss and treatment. Patients were divided into two groups based on whether they had lost <10% of their initial weight in the prior 1-year study or ≥10% (resulting in a 2 ×

Table 1. Patients' characteristics before randomization to sibutramine or sibutramine plus orlistat for the 16-week continuation study

Variable	Sibutramine plus placebo ($N = 17$)	Sibutramine plus orlistat ($N = 17$)
Age (years)	44.3 ± 10.4	43.9 ± 10.7
Weight (kg)	90.1 ± 14.4	88.7 ± 13.5
Height (cm)	163.6 ± 5.4	161.3 ± 10.1
BMI (kg/m^2)	33.6 ± 4.8	34.2 ± 5.1
Age of onset of obesity (year)	17.6 ± 10.5	14.1 ± 9.3
First year weight loss (kg) on sibutramine alone	9.8 ± 8.5	13.4 ± 9.7

There were no significant differences among groups on any of the above variables.

2 ANOVA). (The mean loss for the 16 patients in the first group was 3.3 ± 3.2%, whereas that for the 18 participants in the second group was 18.9 ± 5.8%.) Patients who had reduced <10% during the earlier trial lost 1.2 ± 3.2 kg during the 16-week continuation study, independent of which medications they received. By contrast, those who had lost >10% of weight in the prior trial gained 1.7 ± 2.6 kg during the 16-week study, yielding a significant difference between groups ($p < 0.01$). Figure 2 shows that women who had lost <10% of weight in the prior 1-year trial tended to lose more weight in the continuation study if assigned to orlistat plus sibutramine rather than to sibutramine alone (−2.6 ± 4.9 kg vs. −0.4 ± 1.2 kg, respectively). The difference, however, between conditions was not statistically significant.

A final subanalysis examined weight change in eight women who were assigned to the combination of orlistat plus sibutramine and had lost 5% to 14% of initial weight in the prior 1-year study. These women were selected, because all had responded to sibutramine (i.e., achieved a 5% weight loss) but had not lost so much weight (i.e., ≥15%) as to make further weight reduction unlikely with orlistat. These participants lost an average of 8.4 ± 4.4% of initial weight in the 1-year trial. In the 16-week continuation study, their mean weight increased by 0.2 ± 5.1 kg. Thus, even in highly selected patients, who were thought to be the most likely to benefit from combination therapy, adding orlistat to sibutramine did not increase weight loss.

Medication Dose

Of the 17 patients assigned to sibutramine plus placebo, 7 took 10 mg/d of sibutramine and 10 received 15 mg/d. In the sibutramine plus orlistat group, 6 took 10 mg/d of sibutramine while the other 11 participants received 15

Table 2. Summary of attrition for eight patients

Treatment condition	Reason for discontinuation*	Week	Weight change (in kg) at attrition
Sibutramine†	Lost to follow-up; dissatisfied with treatment	53	−1.0
Sibutramine	PCP removed due to BP: 157/88 mm Hg	56	+0.7
Sibutramine	Premature atrial contractions (found to be an unreported pre-existing condition)	64	+3.8
Sibutramine	Lost to follow-up; dissatisfied with treatment	56	−1.7
Sibutramine	Death in family	56	−1.8
Sibutramine + orlistat‡	Bronchitis and flu requiring hospitalization	64	+5.5
Sibutramine + orlistat	Lost to follow-up; dissatisfied with treatment	64	+0.8
Sibutramine + orlistat	Medical illness in family	53	−0.8

* PCP, primary care physician; BP, blood pressure.
† Sibutramine; mean number of weeks attended was 57 ± 4.1; mean weight change (in kg) at attrition was +0.0 ± 2.4.
‡ Sibutramine + orlistat: mean number of weeks attended was 60.3 ± 6.4; mean weight change (in kg) at attrition was +1.8 ± 3.3.

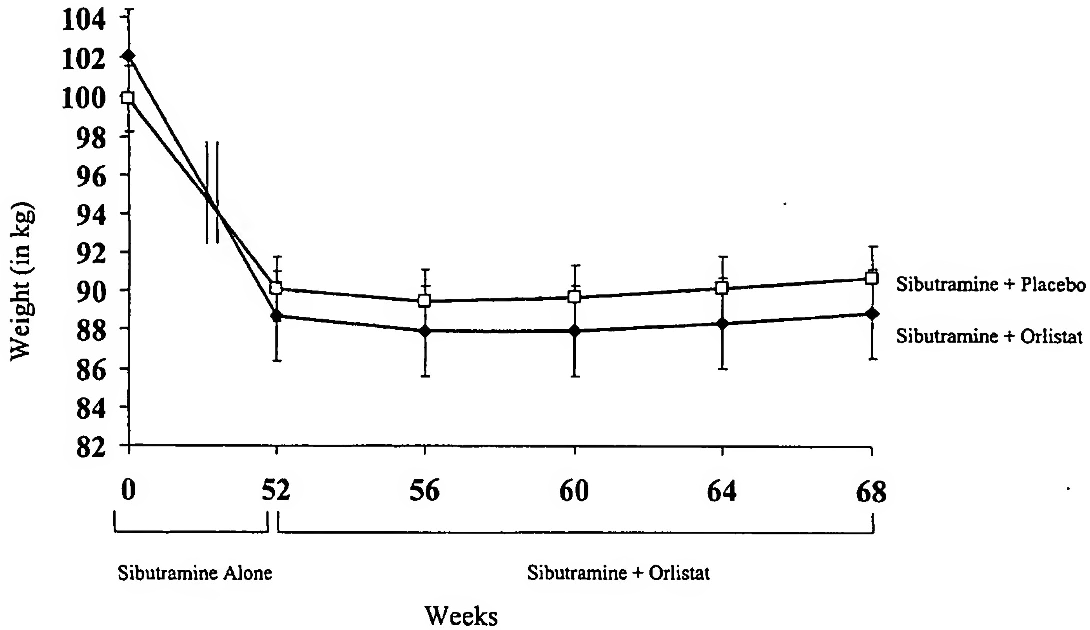

Figure 1. Change in body weight during the 16-week continuation trial for patients assigned to sibutramine plus placebo (N = 17) or sibutramine plus orlistat (N = 17).

mg/d. All patients had been prescribed the 15 mg/d dose in the original 1-year study but, by the end of the year, it had been reduced to 10 mg/d in 13 of 34 women to control side-effects that included insomnia and increased blood pressure and pulse. These reductions occurred before patients began the 16-week continuation trial. There were no significant differences in weight change during the 16-week trial between patients who received the 10 mg/d vs. 15 mg/d dose.

Determination of Treatment Condition

Of the 14 patients assigned to sibutramine plus orlistat, 12 correctly identified their treatment condition at the end of the trial, as did 10 of 12 assigned to sibutramine plus placebo. A chi square test revealed that the percentage of correct identifications (84.6%) was significantly ($p < 0.05$) greater than that expected by chance. Thus, patients appeared to know whether they had received orlistat.

Symptom Reports

Table 4 presents patients' reports of gastrointestinal symptoms during the last week of the trial. Fifty percent of patients treated by combined therapy reported experiencing soft stool and increased frequency of bowel movements at least 1 day of the week, as compared with only 9.1% of patients treated by sibutramine alone. Similarly, 42.9% of

Table 3. Change in weight (kg) for patients in two conditions

	Sibutramine plus placebo		Sibutramine plus orlistat	
Time	EPA*	LOCF†	EPA	LOCF
Week 56	−0.7 ± 1.3	−0.7 ± 1.2	−0.9 ± 1.9	−0.7 ± 1.8
Week 60	−0.3 ± 1.6	−0.5 ± 1.4	−0.7 ± 3.1	−0.7 ± 2.9
Week 64	+0.2 ± 1.9	+0.1 ± 1.8	−0.6 ± 4.4	−0.4 ± 4.2
Week 68	+0.8 ± 2.0‡	+0.5 ± 2.1§	−0.3 ± 4.2¶	+0.1 ± 4.1‖

* EPA = end-point-analysis; N = 16, 10, 12, and 12 at weeks 56, 60, 64, and 68, respectively, for sibutramine plus placebo; N = 14, 15, 15, and 14 at weeks 56, 60, 64, and 68, respectively, for sibutramine plus orlistat.
† LOCF = last-observation-carried-forward analysis; N = 17 for both treatment conditions at all times.
Note: 95th percentile confidence intervals = ‡2.1 to −0.5, §1.6 to −0.5; ¶2.2 to −2.7, and ‖2.2 to −2.0.

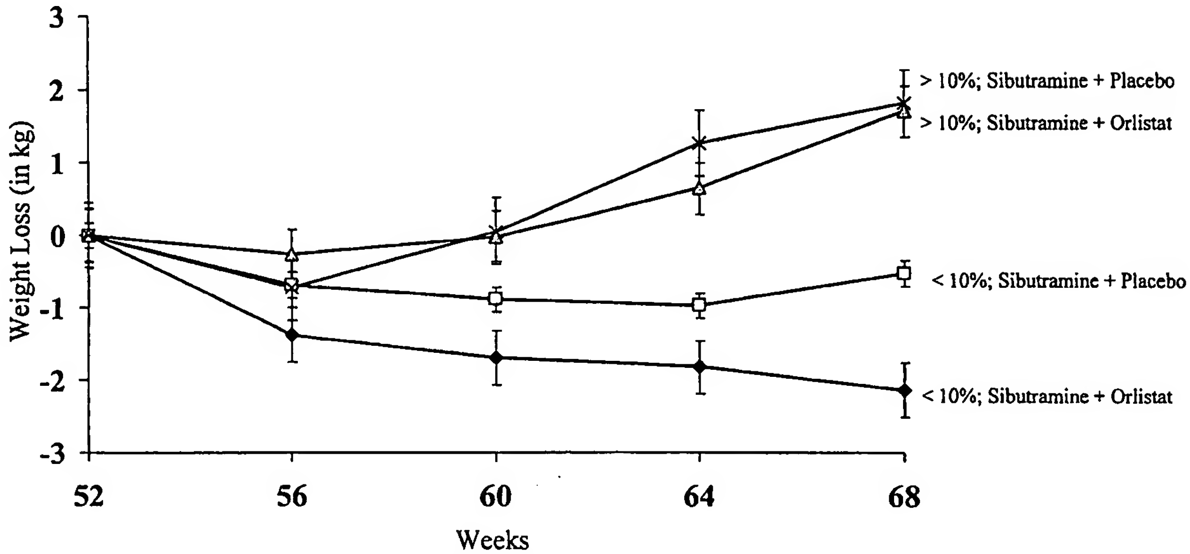

Figure 2. Change in body weight (from week 52) for patients who had lost <10% of initial weight in the prior 1-year trial and were assigned to sibutramine plus placebo ($N = 9$) or to sibutramine plus orlistat ($N = 7$). Data are also shown for patients who had lost >10% of initial weight in the prior trial and who received sibutramine plus placebo ($N = 8$) or sibutramine plus orlistat ($N = 10$).

combined-therapy patients reported oily evacuation and fecal urgency at least 1 day of the week as compared with 0% and 9.1%, respectively, of patients treated by sibutramine alone. Although three of these four differences were statistically significant at the 0.05 level, none was significant at the 0.004 level, the level required if Bonferroni's correction for multiple tests were used.

During physician visits, patients did not report any unusual symptoms that could not be attributed to either sibutramine or orlistat alone. Thus, combining the two medications did not appear to result in any unexpected side-effects.

Discussion

This study's principal finding was that adding orlistat to sibutramine did not significantly increase weight loss in obese women who had previously lost 11.6% of initial weight during 1 year of treatment by sibutramine alone. The two medications did not appear to have additive effects, a finding that disappointed several patients who had hoped, as we had, that they could lose approximately 10% of weight with the first medication and then an additional 10% with the second. The data revealed a trend for patients, who in the prior 1-year trial had lost less than 10% of their initial weight with sibutramine, to lose additional weight by also taking orlistat. However, their loss of only 2.6 kg at the end of 16 weeks was modest and did not differ significantly from that of patients who received sibutramine plus placebo. In addition, it is possible that these patients would have lost 2.6 kg if treated by orlistat alone (without combining it with sibutramine).

Patients who had lost ≥10% of initial weight in the prior 1-year trial appeared to receive little benefit from combined therapy; they gained 1.7 kg during the 16-week continuation study, as did patients treated by sibutramine alone. This finding suggests that there may be limits to the amount of weight that most obese individuals can lose (and maintain) with currently approved medications (8), as well as with behavioral interventions (12). This limit appears to be 10% to 15% of initial weight. Efforts to push beyond this limit may be thwarted by a toxic environment (13) that discourages physical activity while encouraging consumption of a high-fat diet, as well as by compensatory biological responses (14,15) that decrease energy expenditure. Whether alone or together, these factors appear to return weight toward the 10% mark, if not toward baseline (16–18). Andersen et al. (19), for example, used a very low calorie diet to induce an average loss of approximately 15% of initial weight but found that patients maintained a loss of only 10% at the end of 1 year, despite their receiving 30 mg/d dexfenfluramine throughout the trial. Hill et al. (20) similarly found during a 1-year follow-up that patients regained about one quarter of their 11% reduction in initial weight, despite receiving orlistat (120 mg TID) for the full follow-up period. From this perspective, it is not surprising that our most successful patients, who had lost an average of 18.9% in the prior 1-year trial, tended to gain weight in the 16-week continuation study, whether they received sibutramine alone or sibutramine plus orlistat. Even when a subanalysis was conducted on eight women who had lost a mean of only 8.6% of initial weight in the prior trial, they were

Table 4. Patients' report of side effects at week 68

	% of patients endorsing symptoms		
Symptom*	**Orlistat**	**Placebo**	***p* value**
Soft stool	50	9.1	0.04
Increased bowel movement	50	9.1	0.04
Fecal urgency	42.9	9.1	0.09
Oily evacuation	42.9	0	0.02
Oily spotting	28.6	9.1	NS*
Flatus with discharge	28.6	0	0.10
Fatty oily stool	28.6	0	0.08
Liquid stool	14.3	9.1	NS
Stomach pain upset stomach	14.3	9.1	NS
Fecal incontinence	7.1	0	NS
Decreased bowel movement	7.1	0	NS
Pellets/hard stool	7.1	18.2	NS

* NS, not significant.

found to gain 0.2 kg during the 16-week continuation study while receiving sibutramine plus orlistat.

Future medications, or combinations of medications, may well be capable of inducing and sustaining larger weight losses (8). This was the promise of the fenfluramine-phentermine combination until the fenfluramines were removed from the market in 1997 because of their association with valvular heart disease (21).

Results of the present study must be interpreted with caution because of our small sample size. Clearly, further studies are needed that have adequate power to detect clinically significant differences. In designing our investigation, we estimated that patients treated by orlistat plus sibutramine would lose 3.0 ± 3.0 kg during the 16-week trial, whereas those who received sibutramine alone would have a mean weight change of 0.0 ± 3.0 kg. With a sample size of 34, the power to detect this difference was 0.81 ($\alpha = 0.05$, two-tailed test). We thought that, even with this small sample, we would be able to detect at least a trend toward significant differences between the two conditions.

In addition to increasing the sample size (and including men), investigators may wish to use alternative study designs such as comparing sibutramine (plus placebo) to orlistat (plus placebo) to the two medications combined (i.e., sibutramine plus orlistat). There are also a variety of options for sequencing the medications that include prescribing both from the outset or introducing the second medication only after the patient has met a weight-related criterion such as a 5% loss, a 2-month weight loss plateau, or significant weight regain. In addition, longer trials (≥1 year) will be needed to determine whether combined therapy improves the maintenance of weight loss. The present study was limited to 16 weeks, because we were interested primarily in whether adding orlistat to sibutramine would induce further weight loss. Maximum weight loss with medication typically occurs in the first 16 to 26 weeks (8).

We intentionally used a modest behavioral intervention in the continuation study to reveal most clearly the effects of the medications. Use of a more intensive lifestyle intervention may well have increased the size of the weight losses produced by combined therapy (as well as by sibutramine alone), as shown in a previous study (11).

The present findings raise questions about whether it is possible to conduct truly blinded evaluations of orlistat. At the end of the 16-week trial, all but 4 of 26 patients correctly identified their treatment condition. We suspect that the gastrointestinal side-effects associated with orlistat enabled patients to discern their treatment assignment. The problem, however, of patients becoming "unblinded" is not unique to orlistat. Nearly two decades ago Brownell and Stunkard (22) showed that 71% of patients correctly identified whether they had been assigned to fenfluramine or placebo.

In summary, results of this pilot study revealed little benefit of adding orlistat to sibutramine in patients who had previously lost 11.6% of initial weight on sibutramine. Additional studies, however, that include larger sample sizes, as well as different experimental designs, are needed to reach definitive conclusions about the possible benefits of combining these two medications.

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